CURING GENETIC DISEASE WITH GENE THERAPY

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ABSTRACT

Development of viral vectors that allow high efficiency gene transfer into mammalian cells in the early 1980s foresaw the treatment of severe monogenic diseases in humans. The application of gene transfer using viral vectors has been successful in diseases of the blood and immune systems, albeit with several curative studies also showing serious adverse events (SAEs). In children with X-linked severe combined immunodeficiency (SCID-X1), chronic granulomatous disease, and Wiskott-Aldrich syndrome, these SAEs were caused by inappropriate activation of oncogenes. Subsequent studies have defined the vector sequences responsible for these transforming events. Members of the Transatlantic Gene Therapy Consortium [TAGTC] have collaboratively developed new vectors that have proven safer in preclinical studies and used these vectors in new clinical trials in SCID-X1. These trials have shown evidence of early efficacy and preliminary integration analysis data from the SCID-X1 trial suggest an improved safety profile.

INTRODUCTION

Genetic therapies using viral vectors have increasingly been shown to have efficacy in monogenic diseases of children. For successful gene therapy, it is essential to develop a product that allows an effective transduction of target cells. Currently, this is accomplished by using cloned recombinant viruses to transfer genetic sequences with high efficiency (1). In addition, it is critical to ensure proper expression of transgenes in those cells where it is physiologically needed. Increasingly, newer vectors use chimeric promoters of mammalian genes combined with endogenous cis-regulatory elements. Finally, the process must allow for long-term engraftment of modified gene (transduced) cells. Some of the most successful gene therapy approaches to date use *ex vivo* manipulation and hematopoietic stem cell (HSC) transplantation as a clinical platform to effect genetic therapies (2).

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The strategy for *ex vivo* modification of HSC is remarkably similar to that developed in the early 1980s (1, 3), with some important improvements. Generally, HSCs are obtained using either bone marrow harvest, mobilized peripheral blood collection, or, less frequently, autologous umbilical cord blood. An HSC-enriched population of cells is obtained using CD34 isolation. The resulting CD34+ cells are then incubated *ex vivo* in a cocktail of cytokines and subsequently exposed to a safety-certified virus vector supernatant manufactured in specialized facilities according to good manufacturing practice (GMP) guidelines. The transduced cell product is then administered as an autologous hematopoietic stem cell transplant (HSCT) to the recipient. In some protocols, the recipient is exposed to preparative conditioning using chemotherapy, radiation, or both as per usual HSCT transplantation protocols. In some protocols, no conditioning is required and these details are disease-specific. Two major advantages are attained using this gene therapy approach: 1) there is no need to search for a histocompatible donor; and 2) there is no risk of graft-vs-host disease (GVHD) and therefore no need for GVHD prophylaxis or treatment of the patient.

As noted above, one candidate disease for genetic therapy is X-linked severe combined immunodeficiency (SCID-X1) (4). The disease is caused by loss-of-function mutations of the interleukin (IL) – 2 common gamma (γ) chain cytokine receptor. Phenotypically, children born with this disease lack T and natural killer (NK) lymphocytes and have poorly functioning B cells leading to severely compromised immunity. The disease is fatal if untreated, often from otherwise relatively benign viral infections. Previous clinical work has shown that allogeneic HSCT using either matched related or matched unrelated donors (MUDs) can cure the disease often without any conditioning of the recipient. However, MUD transplantations in this disease are accompanied by increased risk of GVHD, graft failure and overall poor outcomes of these transplants, particularly when the recipient is infected at the time of transplantation. This is frequently the case, as patients are frequently diagnosed due to serious viral infections in the first year of life.

Previous trials have shown effective gene therapy in SCID-X1. Two trials treated a total of 20 children with this disease using a Moloney leukemia virus (MoLV) – based retrovirus vector expressing the IL-2Rγ cDNA from the viral long-terminal repeat (LTR) cis-regulatory element encompassing a strong enhancer element and a viral promoter (MFG-γC) (5, 6). In these previous studies, efficacy was shown in 18 of 20 children treated with this vector with a return of T and NK cell numbers and functions. However, 5 of 20 children in this trial devel-

oped T cell leukemia related to the insertion of the viral vector into the genome near proto-oncogenes (7) These insertions led to dysregulated expression in four of five cases of the *LMO-2* proto-oncogene implicated in some cases of de novo childhood T cell acute lymphoblastic leukemia (8) Of these children, 4/5 were successfully treated for their leukemia with maintenance of the gene-corrected immunologic function, whereas one child died from therapy-resistant leukemia (9). Thus, although showing efficacy, these studies were also characterized by serious adverse events (SAEs) that led to the interim cessation of a number of trials worldwide and to the United States Food and Drug Administration (FDA) restriction of the use of gene therapy in SCID-X1 to rescue protocols in which eligibility would require previous failure of an allogeneic transplant.

MATERIALS AND METHODS

Shortly after the development of leukemias in the French SCID-X1 gene therapy trial, a group of investigators established the Transatlantic Gene Therapy Consortium (TAGTC) to promote a collaborative approach in addressing the SAEs in this trial (10). This group began meeting annually in a planning retreat with goals to: 1) share expertise in addressing the SAEs of the gene therapy trial in SCID-X1; 2) collaborate on vector development and preclinical studies; 3) share the costs of GMP vector production and certification; 4) develop a common clinical protocol as a platform for a multi-institutional trial and subsequently implement this trial across multiple sites; and 5) seek funding for these efforts.

Vector design was based on the molecular data implicating the MoLV U3 region enhancer in trans-activation of *LMO2* locus. Preclinical efficacy and safety data were generated at multiple sites using a variety of *in vitro* and *in vivo* assays with both murine and human cell lines and primary cells. These data ultimately contributed to the development of regulatory documents needed in the United States (US), United Kingdom, and France for opening of a clinical phase I/II trial. The requirements for each of these regulatory processes varied according to specific governmental protocols. Vector GMP production was accomplished at one site using transient transfection methods and has previously been reported (11).

RESULTS

The institutions making up TAGTC are shown in Table 1. The vector proposed and subsequently used in the trial, SRS11-IL2RG, is shown

TABLE 1
Transatlantic Gene Therapy Consortium

Children's Hospital Boston, Harvard Medical School (Boston)
CIEMAT (Madrid)
Cincinnati Children's Hospital, U. of Cincinnati College of Medicine (Cincinnati)
Genethon (Paris)
Georg-Speyer-Haus (Frankfurt, Germany)
German Cancer Institute (Heidelberg)
Great Ormond Street, Institute for Child Health (London)
Hannover Medical School (Hannover)
Lund University (Sweden)
Mattel Children's Hospital, UCLA (Los Angeles)

in Figure 1. The vector is a self-inactivating (SIN) designed γ-retrovirus in which the U3 enhancer is deleted from the 3' LTR. In this SIN design, upon reverse transcription of the viral genome, the 3' LTR is duplicated such that the integrated provirus is devoid of both 5' and 3' U3 enhancer sequences. A variety of preclinical studies were used to compare this vector to the MFG-γC vector used in the previous trial, including: 1) efficacy in restoring IL2R signaling *in vitro*; 2) reduced propensity for immortalization of primary murine bone marrow *in vitro* (12); 3) reduced transactivation of the LMO2 locus in a plasmid reporter assays in Jurkat T cells (13); 4) reduced insertions in proto-oncogenes in mice transplanted with transduced HSC (manuscript in preparation).

In the United States, extensive reviews by the National Institutes of Health Recombinant DNA Advisory Committee, the FDA, local international review boards, and a study section of the National Institute of Allergy and Infectious Diseases (NIAID) led to multiple — often conflicting — recommendations for changes in the protocol. The resulting approved protocol "Gene transfer for SCID-X1 using a self-inactivating (SIN) gamma retroviral vector, a multi-institutional

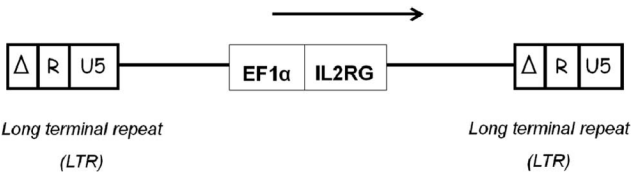

FIG. 1. The SRS11-IL2RG vector. The vector was derived from a murine Moloney Leukemia virus (MoLV). The long terminal repeat (LTR) includes a deletion of the U3 region denoted by Δ, the R and U5 regions are shown. The IL-2 receptor γ cDNA (IL3RG) is expressed from an internal mammalian promoter made up of the elongation factor 1α (EF1α) sequences. *Arrow* denotes orientation of mRNA generated from integrated vector.

phase I/II trial evaluating the treatment of SCID-X1 patients with retrovirus-mediated gene transfer" was ultimately approved by regulatory agencies for opening in five sites internationally (Table 2) and is listed on Clinicaltrials.gov. The trial is funded in the United States by the NIAID.

The trial opened for enrollment at the US sites in January 2011 and to date has enrolled ~50% of its accrual target (nine patients). The longest followed subjects are now >3 years since infusion of gene modified cells. There have been no SAEs directly related to the vector or to insertional events. Analysis of T cells in multiple subjects show expression of IL-2RG on surface of T cells at levels slightly below wild-type T cells, as expected based on the weaker nature of the promoter. Efficacy studies are underway, but to date the vector appears to perform as expected with a number of subjects showing return of peripheral T cell counts and T cell functions as measured by *in vitro* stimulation index and return of NK cell numbers. Tracking of recent thymic immigrants via T cell receptor excision circles confirm the functioning of the thymus in many subjects. Integration studies are ongoing.

DISCUSSION

Collaborative work across multiple institutions making up the TAGTC has led to the development of an enhancer-deleted vector expressing the IL-2RG. In preclinical studies, the vector showed improved safety profile using a variety of *in vitro* and *in vivo* assays. This represents a unique international collaboration with shared costs, developmental work, and subsequent clinical trial. The clinical trial, still ongoing and accruing additional subjects, shows early efficacy. Although the follow-up is still too early to assess overall safety, no SAEs have yet been identified related to the vector.

These studies are an example of so-called two-way translational research in which basic science initially leads to clinical research studies, the results of which then lead to new rounds of basic studies and subsequent trials. The field of genetic therapies has continued to mature over ~25 years in a fashion analogous to many new technol-

TABLE 2
Participating Institution Sites in SCID-X1 Clinical Gene Therapy Trial

Great Ormond Street, Institute for Child Health (London)
Hôpital Necker Enfants Malades (Paris)
Children's Hospital Boston, Harvard Medical School (Boston)
Cincinnati Children's Hospital, U. of Cincinnati College of Medicine (Cincinnati)
Mattel Children's Hospital, UCLA (Los Angeles)

ogies in which initial successes are followed by a focus on side effects with subsequent new advances occurring before broad acceptance and applications. In this case, the use of viral vectors for gene therapeutic purposes showed initial successes in the late 1990s and early 2000s. Vector insertional mutagenesis led to a dampening of enthusiasm followed by investigations to improve safety, as described in this manuscript. The field has recently seen multiple successes in treating rare, monogenic diseases using new vector technologies. Ongoing research will be aimed at broadening the indications and extending the applications beyond highly specialized academic research centers.

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DISCUSSION

Longo, Boston: David, that's very encouraging for the new changes in the vector. I have two questions. It looked as though the CD4 plus CD8 total didn't equal the CD3 level. Are you seeing CD4/CD8-negative T cells being generated? That's question one. Question two is, are you following treks and looking at the repertoire to see whether or not you are getting a good representation?

Williams, Boston: I'll answer the second question first. We are following treks and we are also molecularly following T cell receptor repertoire, and in both cases it looks like we have robust immunity from a diversity standpoint. To answer the first question, we do see an actually interesting increase in gamma/delta T cells in the beginning; and, talking to our transplant colleagues, this isn't all that uncommon post-transplant in kids with SCID disease — that the gamma/delta fraction appears to expand quickly first. And we are actually now using that in a new trial to look at the effects of specifically depleting alpha/beta — maintaining gamma/deltas — in haploidentical transplants to try to increase anti-infectious immunity but reduce graft-versus-host disease.

High, Philadelphia: David, if these exciting results continue, can you tell us what you think will be the approach to X-linked SCID going forward for newly diagnosed patients?

Williams, Boston: That is a good question. So many people in the audience may not know, but SCID is now a disease that there is newborn screening for in the United States — increasingly, but not all states. And this actually does raise an interesting issue, which is, if you have a newborn child with no infections, what's the right approach; whether it's a haploidentical transplant, allotransplant, or gene therapy. The results that I've shown you so far in this trial, plus the previous trial of 20 patients, suggests that this approach is at least equally efficacious as an allotransplant and has, overall, a different risk profile, but the same numbers of adverse events, in general. So I think our approach has been that if there is an HLA-identical sibling, we would still go directly to a transplant from a sibling donor. But if there's not, then we offer families this option, as well as an allotransplant option explaining to them the side effects of each, which are somewhat distinct.

Reddy, Ann Arbor: Any thoughts on what precipitated the inflammation in that one particular patient, and did you see such inflammatory excesses in any of the other eight patients?

Williams, Boston: I probably went so quick it was hard to understand this point. This child had non-typeable viral ulcers that were resistant to antiviral therapies. And the inflammation that we saw was coincident with the development of NK cells from the transduced stem cell products. So the infiltration basically was natural evolution of the replacement of his defective immune system with an infective immune system and led to the resolution of these ulcers. And we have seen that in other children who've had medically resistant viral infections that resolve, including, for instance, those in patients

from other parts of the world who get BCG vaccinations. Just to go back to you, Kathy, one thing I would say is that these results are actually at a level that we've now been approached by two biotech companies wanting to take these results into registration trials for orphan disease. So I think when you get companies interested in something like this, then it starts to seem like the results are good enough to offer as therapies in the future. At least that's the way they are looking at it.